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OBSERVATIONS ON THE MARROW OF THE BONE AND THE SPLEEN IN A CASE OF LEUKÆMIA.

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Time has not permitted a complete study of the present case of leukæmia. In the following paper attention will be called to some observations made upon the structural changes in the marrow and in the spleen and to the bearing they may have on the process of cytogenesis. Unfortunately, it has not been possible to study this process in the normal organs from the point of view suggested in the paper.

The case was one of leukæmia, treated in the Philadelphia Hospital under the care of Dr. William E. Hughes. The case was diagnosed as one of splenic and myelogenic leukæmia.

The notes of the *post-mortem* are as follows:

Isaac L., aged fifty-five years, white, male, native of England, admitted to the Philadelphia Hospital, December 11, 1894; died February 5, 1895. Post-mortem made sixteen hours after death.

Pathological Diagnosis. Anæmia. Hyperplasia of the spleen. Lymphoid marrow of the bones. Lymphoid infiltration of the liver and the kidney.

Body of a very emaciated man. Surface of the body considerably discolored by freezing, but showing evidences of a slight yellow discoloration. Round, superficial ulcers are found over the external malleoli.

Abdominal Cavity. The liver projects beyond the edge of the thorax 4 cm. in the right mammary line, 10 cm. in the median line, and 4½ cm. to the left of the same. The spleen projects 9½ cm. in the left mammary line, and extends 2½ cm. beyond the median line. The left lobe of the liver overlaps the spleen 1 cm. The appendix projects inwardly, and then bends down over the psoas muscle into the pelvis.

Thoracic Cavity. The lungs are free and small, and the pleural cavities are empty. The costal pleura is marked by minute spots of dark pigment in the intercostal spaces. A few of these are found also in the mediastinal pleura. On opening the pericardial sac a dense band of adhesion is found in front.

The right ventricle is covered by a large white fibrous patch. The rest of the visceral pericardium shows evidences of myxomatous change.

Heart. Both ventricles are flabby. The left cavities contain a small amount of liquid, pale-rose-colored blood and some grayish coagula. The mitral orifice admits two fingers. The right cavities contain a large, soft, grayish clot and a small amount of liquid blood. The tricuspid orifice admits with difficulty three fingers. The right auricle is somewhat dilated. The other cavities are normal in size. The mitral valve is slightly thickened and presents a few opacities. The other valves are normal. The apices of the papillary muscles show some evidences of fibroid change. The aorta presents some patches of atheroma near the valves. The myocardium presents a brownish color. The orifice of the pulmonary artery measures 10½ cm. in circumference. The pulmonary valves appear somewhat large. The aortic orifice measures 9½ cm. in circumference. The heart weighs 310 grammes.

The left lung presents extensive anthracosis. The surface of section is otherwise normal. The bronchial glands are of normal size, and some of them are slightly indurated and surrounded by a thickened capsule. Others are rather soft. They are all black in color. The lung weighs 460 grammes. The right lung similar to the left. The bronchial mucous membrane of both lungs is somewhat swollen, and presents a marble discoloration of a purplish and gray color. The organ weighs 680 grammes.

Spleen. The splenic vein flattened out measures 1.3 cm. in diameter, and the artery half a centimetre. The organ is large, dense, and irregularly tongue-shaped, the upper extremity being thick and rounded and the lower extremity presenting a flattened edge. It presents a large concavity toward the hilum. There is a large notch on the posterior border and a smaller one on the anterior. The organ measures 25 cm. in length, 14 in width, and 10 in thickness. At the upper end there is some thickening of the capsule. The latter presents a pale-magenta color. The surface of the section is smooth, and shows the color of cherry wood. There is no prominence of the Malpighian bodies. Toward the cortex the structure is slightly translucent, and very minute areas of a darker color and slightly translucent are seen throughout the substance. There is no prominence of the trabecula. The organ weighs 1810 grammes.

The suprarenal bodies are soft and dark in color. The connective tissue around them shows evidences of mucoid change.

The left kidney is rather soft, the capsule is easily removed, and the surface is smooth and grayish-brown in color. The surface of section shows a marked contrast between the pyramids and the cortex. The former are of a rose-red color, and the latter is gray. The structure of the latter is not clear. The organ weighs 148 grammes. The right kidney presents a similar appearance to the left, and weighs 148 grammes. They both show some reduction of the cortex. The color of the latter may be compared with that of dark coffee and milk.

The pancreas is somewhat firm, and presents a pale yellowish-gray color. It is otherwise normal.

The stomach is rather contracted. The mucous membrane is smooth and presents a pale-rose color.

The duodenum is normal. The common duct is patulous. The bile is fluid, and of a pale-yellow color.

The liver is rather soft, and the capsule presents a few patches of thickening. The color is of a rather pale brown, presenting in some portions minute mottlings of a yellowish-gray color. The organ measures transversely 25 cm. The right lobe measures antero-posteriorly 22 cm., and the left lobe 19. The right lobe is 9 cm. thick. The surface of section is smooth, brown, and marked by minute grayish areas in the periphery of some of the acini. The organ weighs 1900 grammes.

The mesentery contains a small amount of fat showing mucoid change. The mesenteric glands are very slightly enlarged, of normal consistency, and some of them on section present a marbled appearance of white and rose color. The inguinal glands are similarly affected. No change is found in other lymphatic glands.

Intestines. The valvulæ conniventes are prominent and œdematous. In some places they are stained with bile, and in others they show evidences of congestion. The large intestine is normal.

The abdominal aorta is large and presents a few patches of atheroma, especially in the lower portion. It contains puriform clots. The thoracic duct presents a normal caliber and rather thin walls.

The testicle is rather soft, and presents striæ of a yellowish-brown and rose-red color,

The bladder is normal.

Bones. The sternum presents a brownish-purple and pulpified marrow. The costal cartilages present on section a marked translucent brownish central area. The medulla of the femur is rather soft and dark purple in color.

The sections of the marrow and the spleen that have received careful attention in this case were cut from small pieces that were placed for two hours in Müller's fluid, then washed, fixed in Fleming's, hardened in alcohol, and cut in celloidin. The methods of staining were safranin, Benda's hæmatoxylin stain, which was used for tissue that had been hardened in Müller's fluid, Biondi's fluid, and a stain of safranin, orange G, and methyl-blue.

Anyone undertaking the study of these two organs recognizes at once the difficulty of the problem before him. The variety of opinions expressed as to the nature of the cellular elements is rendered more confusing by the variety of methods that are suggested for their study.

No attempt will be made in the present paper to enumerate and discuss these opinions. In the bone-marrow especially we are confronted with cells that are diplasmatic to a marked degree. We have here in the loose reticulum, which has a tendency to arrange itself in the form of tubes, cells of varying size. Some of them contain fat, others hæmoglobin, others eosinophile granules, others present an osseous protoplasm, and others the colorless protoplasm of the amœbocytes. A rather ineffectual attempt to dissociate these cells is based upon peculiarities of the nucleus, even before the protoplasm becomes characterized by the different forms of metaplasma. The manner of division of the nucleus is insisted upon as an important feature in the differentiation of these cells. It is very probable, however, that different forms of nuclear division may be found in cells that are not fundamentally distinct from one another.

In the present case the lymphoid reticulum of the bone-marrow is found slightly thickened, and spaces are crowded with a variety of cells. These may be seen in Figs. 1, 2, and 3, accompanying the present paper. Attention is especially called to Figs. 1 and 2, where different forms of nuclear division and a variety of cells are shown. Nowhere in the marrow has any evidence been found of the formation of red blood-cells. There are, undoubtedly, numbers of cells that present the nuclear features that are said to be characteristic of erythroblasts, but it appears that they become overgrown with protoplasm, or may present evidences of nuclear cell-division without being able to form red blood-globules. The protoplasm of such cells frequently shows unmistakable evidence of the presence of hæmoglobin. In Fig. 1 a widely dilated endothelial channel is shown filled with such cells. Their nuclei present the indirect forms of segmentation of the nucleus that have been described by Arnold. There are evidently no karyokinetic figures. In Fig. 2 a large marrow-cell is seen to the left, presenting a similar polymorphous arrangement of the nucleus. The nucleus of this cell presents a small amount of chromatin and a distinct nuclear membrane. To the right of this we find an erythroblast which shows swelling of the protoplasm and increase in the amount of chromatin and an attempt at mitotic division of the same. Such cells are frequently found in the marrow in the present case, and undoubtedly find their way into the circulation and swell the number of leucocytes and nucleated red blood-cells. The next cell to the right is a resting-cell of the same

kind as the one to the left, but in which no fragmentation is taking place. We have, finally, to the right of this a large fat-cell. In fact, in these wide channels of the lymphoid marrow we may find cells in all stages of direct nuclear fragmentation and varying forms of chromatolysis, and cells varying in size from the small round erythroblasts (that will be described further on) to the large cells shown in Figs. 1 and 2. With the exception, perhaps, of the true erythroblasts, these cells may be considered as interchangeable, and even the erythroblasts appear to be subject to such changes as may bring about their conversion into the form of cell described as leucoblast, that is, their nucleus may become gradually poorer in chromatin, and will assume by degrees the loose reticulated appearance characteristic of the larger cells.

In regard to the spaces where these cells are to be found in the bone-marrow we note the fact that many of them are not lined with endothelial cells. Some of them, on the other hand, present the endothelial lining of capillaries. These endothelial cells show no evidences of nuclear cell-division. They are oval in shape, poor in chromatin, and project slightly into the lumen of the vessel. These endothelial channels appear to be surrounded by channels that are not lined with endothelial cells. It is in these peri-endothelial channels that we shall find, later on, in the spleen, a large number of erythroblasts engaged in the process of red blood-formation. In the marrow of the bone, however, they do not gather in these localities, and therefore fail to contribute to the formation of red blood-cells. This is true at least of the present case of leukæmia.

I wish to call especial attention to the cell marked with the letter A in Fig. 3. The two nuclei represented in this cell are surrounded by a transparent zone. The nuclei are homogeneous and stain intensely red with safranin. It will be seen later on that bodies similar to these nuclei are especially concerned in the formation of red blood-cells. Cells similar to the one marked with the letter A are found containing even a larger number of nuclei. These may be separated, as in the present instance, or they may be joined together by filamentary prolongations. The protoplasms around them contain hæmoglobin. These cells undoubtedly represent ineffectual attempts at the formation of red blood-cells. As will be seen, when we come to study the spleen, the successful formation of erythrocytes seems to take place

only from free nuclei similar to those found within this mass of protoplasm. It will be seen, furthermore, that the successful formation of red blood-cells takes place only in contact with endothelial cells, and, in the present case, only in the spleen. We should not fail to notice the marked difference that there is between the cell at present described and the one containing pale nuclei. The nuclear filaments that may be found joining the fragments of the nucleus in the latter cells are pale and usually thick, whilst the filaments of the multinuclear erythroblasts are stained intensely red and are very fine. I should mention here that when the triple stain of safranin, orange G, and methyl-blue is used, the pale nucleus of the so-called leucoblasts takes a greenish color, whereas the homogeneous nucleus of the erythroblasts, whether found free or within the protoplasm of large cells, takes the red stain of the safranin. In fact, the orange G acts as a decolorizing agent, removing the safranin stain from all the structures except the erythroblastic nuclei. In the upper part of Fig. 3 a few cells are shown presenting the greenish stain in contrast with a few homogeneous small nuclei stained red. The appearance of structure in some of these red nuclei will be noted later on.

We find also in the bone-marrow a large number of cells containing a large vacuole and presenting a few granules of fat in the protoplasm around the vacuole. These are evidently fat-cells from which the infiltrated fat has been removed.

Red blood-cells of various shapes are found in the channels of the marrow together with the cells above described. Many of them are very pale, and some of them are nucleated red blood-cells. We find nowhere any clear evidence of pigmentary degeneration of these cells.

In the spleen we find a general increase of the connective-tissue reticulum. We find here, as in the marrow, a number of parallel channels, some of them lined with endothelium and others not. The latter are frequently crossed by fibrillar prolongations of the reticulum. The nodes of the latter present here and there stellate connective-tissue cells and occasionally a flat endothelial cell with slightly projecting pale oval nucleus. These spaces contain cells similar to those that have been described in the bone-marrow. The number of red blood-cells, however, is greater, as is also that of the free nuclei that we shall describe here as true erythroblasts. These are most

numerous in the immediate vicinity of the channels that possess a distinct endothelial wall. These will be considered as capillaries. The point that I wish particularly to call attention to is the behavior of the erythroblasts in the immediate neighborhood of the endothelial channels. It is here that we see for the first time anything like the formation of red blood-globules. The process is something as follows:

The small free erythroblast sends a prolongation of varying length through the endothelial wall from without toward the interior of the vessel. This prolongation just within the endothelial lining swells into a bud which may be smaller or larger than the erythroblast from which it springs. This small bud presents the same peculiarities as the erythroblast outside. It is around this bud that we see the formation of the red blood-cells. These seem to grow around the bud as a protoplasmic formation around the nucleus, except at the point of contact with the filament. If the bud presents a round shape, the hæmoglobin-holding protoplasm is also globular. If the bud is pear-shaped, the protoplasm generally presents the same outline. Occasionally the protoplasm grows more from one side of the nucleus. The union between the erythroblast and the bud may be very close, presenting the appearance of a large diplococcus divided by a line of endothelial membrane. In other cases the filamentary union may be as long as 9μ. In some cases the bud has evidently been broken off, or has not formed, and the end of the filament can be seen pointing in the endothelial wall. When the bud-cells, with their hæmoglobin envelope, are disengaged from the endothelial wall they constitute a nucleated red blood-cell. As some of these nuclear buds are found of very small size, with a large blood-cell around them, it is very probable that the latter may be loosened from its anchorage without any nuclear contents, thus constituting a fully developed red blood-cell. There is also evidence that the bud may be loosened from its moorings without any hæmoglobin formation about it. In this case it constitutes, it appears to me, a true blood-plaque. The measurements of one of these buds gives for the red homogeneous nucleus within the endothelial channels 3μ in diameter, the red body outside of the endothelial channel measures 2μ in diameter, the filament between them 1μ, and the hæmoglobin capsule $5\frac{1}{2}\mu$. Another one in the same capillary presents a bud of $1\frac{1}{2}\mu$ in diameter and a hæmoglobin capsule of 6μ. The filament penetrates the endothelial wall for a distance of 2μ. Here it has evidently

broken off in the preparation. The outline of the hæmoglobin envelope is generally globular, and the protoplasm is slightly granular and yellow. In another capillary we find the following evidences of budding. First, a homogeneous red body $3\frac{1}{2}\mu$ in diameter in close contact with the external surface of the endothelial wall, through which it sends a tip-like projection $1\frac{1}{2}\mu$ long by 1μ wide. This is capped within the blood-vessel by a pear-shaped red blood-cell very slightly larger than the erythroblast without. Next to this there is an endothelial nucleus, and just beyond it another filamentary figure (Fig. 5, a) with the following dimensions: Outside of the endothelial channel there is a round, intensely red homogeneous body. This body measures nearly 3μ in diameter. There is no protoplasm about it. From this body a fine filament, slightly bent upon itself, extends a distance of 6μ to a similar red body just within the endothelial line. The fine filament expands slightly as it goes through the endothelial wall, thus giving something of a pear-shape to the body within the endothelial channel. The latter body is equal in diameter to the one outside. The hæmoglobin capsule springs from one side of this body and is somewhat oval in shape, measuring $3 \times 3\frac{1}{2}\mu$. A small portion of hæmoglobin protoplasm is also seen projecting from the opposite side of the endothelial bud. Further on we find (Fig. 5, b) a pear-shaped red body close to the endothelial lining, with a point imbedded in it. This is continued into a short filament somewhat bent upon itself, and terminates in a similar pear-shaped body within the endothelial channel. Springing from this we have a pear-shaped red blood-cell measuring about 6μ in diameter; next to this there is a closely joined couple of pear-shaped bodies. All these are found upon the same side of the capillary within a distance of 50μ. This process of filamentary budding never takes place through the endothelial nucleus, but through the cement or the protoplasm of the flat endothelial plate.

There are several reasons to support the view that these filamentary buds project from without to the interior of the capillary. They are as follows:

1. We always find an absence of hæmoglobin around the erythroblast outside of the endothelial channel. It is reasonable, then, to suppose that the globule acquires its hæmoglobin envelope after it penetrates the endothelium and comes in contact with the circulating plasma.

2. We find in the majority of instances that the body outside of the endothelial channel is larger than the one within.

3. We find also evidences of progressive chromatolysis in the bud, usually in proportion with the growth of the hæmoglobin capsule. Sometimes there is nothing left but the point of the chromatin filament to which the red blood-cell is attached. The bud further shows evidences of dissolution by breaking up into minute filaments radiating for a short distance from the point of attachment. The latter change gives these corpuscles, with their filamentary tail, the appearance at times of spermatozoa.

The small, round erythroblasts are not always homogeneous. Some of them present within the well-stained nuclear matrix minute granules of a darker color. These are so arranged sometimes as to give the impression of mitotic figures. It is probable that they are such, but it is difficult to analyze them on account of the intense coloration of the matrix and the close packing of the supposed mitons. In other cases we find within the erythroblast fine lines of a lighter color than the surrounding substance. In such cases the chromatic substance appears broken up into four fragments by a pale crucial figure. The erythroblasts engaged in the process of filamentary budding are homogeneous, except that occasionally, as previously mentioned, the bud breaks up into small rays extending a short distance into the red blood-cell. Erythroblasts that are not engaged in the process of filamentary budding are frequently of a larger size. In these a densely packed coil of chromatin may be seen, and sometimes a rearrangement of the miton in obscure karyokinetic figures. I am of opinion that in such cells the nuclear matrix may swell before the nucleus divides, and the cell is thus converted into the so-called leucoblast.

Though the splenic channels, endothelial or non-endothelial, are frequently crowded with red blood-cells of varying shape, we find very little evidence of pigmentary degeneration. The irregular distribution of these red blood-cells makes it difficult to decide upon the nature of the endothelial channels —that is, as to whether they are or are not true capillary continuations of the bloodvessels of the spleen. In my opinion they are; but this question belongs to one of the many difficult problems in the histology of the spleen.

For purposes of comparison, the spleens of a case of cardiac anæmia and of pernicious progressive anæmia have been examined. In neither

of these have the filamentary figures been found. It must be stated, however, that in the case of pernicious anæmia the tissues were not fixed in osmic acid, and that in the case of cardiac anæmia, though the attempt was made to fix the tissues by means of osmic acid, the result was not quite successful, evidently on account of post-mortem changes.

In the spleen of cardiac anæmia the number of red blood-cells in the spleen channels is very great. The endothelial cells show no evidences of division. They are, however, more numerous and more globular than in the cases of leukæmia. They project further into the endothelial channel. Sometimes they are very close together, leaving scarcely any space between them. The closer together they lie the more prominent and globular they appear. These endothelial nuclei measure between 9μ and 12μ in length and 5μ in width. The endothelial channels, besides containing frequently a large amount of hæmoglobin-cells, contain also a few leucocytes. The majority of them are multinuclear, and many of them contain fat-granules in their protoplasm. Occasionally we find an erythroblast nucleus in the interior of a protoplasmic body containing hæmoglobin. These are not found in endothelial channels. Free erythroblasts are also numerous.

In the case of pernicious anæmia the endothelial nuclei are still more swollen and numerous than in the case of cardiac anæmia. The capillary spaces are evidently dilated. In both the spleen and marrow we find distinct evidences of pigmentary degeneration of the red blood-cells. We also find, especially in the marrow, rows of fat-granules in the endothelial lining of the vessels. These rows are generally discrete, and sometimes they bifurcate, leaving the nucleus in the middle. The number of erythroblasts is smaller in these organs than in those of leukæmia. The same may be said of the number of eosinophile cells.

As to the origin of the erythroblasts, the opinion is maintained in the course of the paper that all the cells described may develop one from the other through a species of metaplasia, the starting-point of which is probably the lymphocyte. The erythroblast appears to originate in the shape of spherical fragments of chromatin that are thrown off from the nuclei of other cells; and the latter may again develop from the erythroblasts by a process of chromatolysis and swelling of the nuclear matrix. I do not pretend, however, to insist

upon this point. The opposite view, that gives the erythroblasts a specific character and traces their origin to embryonal red blood-cells, is also well supported.

My object in this paper has been to call attention to the process of filamentary budding of erythroblasts through the endothelial wall as a factor in the formation of red blood-cells. It is very improbable that this process should occur in the leukæmic spleen and not under normal circumstances.[1]

Figs. 4, 5, 6, 7 show capillaries of the spleen in which the process of filamentary budding manifests itself. Fig. 5 has been described in the text. In Fig. 6 a couple is shown in which the endothelial bud is of very small size and shows evidences of breaking up into indistinct filaments within the red blood-cell. This figure shows an arrangement of the parts that is frequently met with, namely, that the endothelial channels are much narrower than the non-endothelial channels around them. Fig. 7 shows a very long filamentary bud. In Fig. 4 a capillary is shown, in the upper border of which three filamentary buds are forming.

Figs. 8 and 9 represent peculiar bodies found in the marrow of the case of leukæmia. It is impossible to account for these either by supposing them to be forms of degeneration of the nucleus or groups of red blood-cells. They resemble somewhat a sporoblast formation. By careful focussing the group is found to consist of nine cyst-like bodies, each one of which measures 3–4μ. They are vesicular in structure and present a rod-shaped body lying near the periphery of the vesicle. The vesicles take a pale reddish-gray color with safranin, and the rod-shaped body stains intensely red. A considerable number of these are found in the marrow of the case of leukæmia. They may be found in groups of twos and threes; the one represented in Fig. 9 and reproduced from a photograph in Fig. 8, is the largest and most distinct of these groups that I have found. It is possible that these may be indications of the so-called "vesicular degenerations" of the nucleus, or that they might be red blood-cells of small size presenting some change in their wall which leads to the appearance of the rod-shaped nucleus. It must be admitted, however, that these explanations are not satisfactory and that the nature of these bodies must be left in doubt until opportunity is given for a more complete investigation.

[1] Since the reading of this paper I have found the same process in the spleen of the rat, and of the rabbit, after hemorrhage.

EXPLANATION OF ILLUSTRATIONS.

FIG. 1. Bone-marrow of leukæmia, large irregular nuclei, with hæmoglobin protoplasm. Compen. oc., No. 4. Apo. oil imm., 2 mm.

FIG. 2. Bone-marrow of leukæmia. *a*. Large polymorphous nucleus with hæmoglobin protoplasm. *b*. Large erythroblasts with imperfect mitotic division of the nucleus. *c*. Fat-cells. Compen. oc., No. 8. Apo. oil imm., 2 mm.

FIG. 3. Bone-marrow of leukæmia. *a*. True erythroblasts surrounded by a mass of protoplasm containing hæmoglobin. Compen. oc., No. 4. Apo. oil imm. 2 mm.

FIG. 4. Capillary of leukæmic spleen, showing the budding of erythroblasts through the endothelial wall. Three specimens are shown in the upper wall. Compen. oc., No. 4. Apo. oil imm. 2 mm.

FIG. 5. Capillary of spleen, described in detail in the paper. *a* and *b* indicate erythroblasts in process of filamentary budding. Case of leukæmia. Project. oc., No. 2. Apo. oil imm., 2 mm.; camera length, 85 cm.

FIG. 6. Section of leukæmic spleen, showing erythroblasts in process of budding. Minute bud within the endothelial channel surrounded by a hæmoglobin envelope. Project oc., No. 2. Apo. oil imm., 2 mm.; camera length, 85 cm.

FIG. 7. Long filamentary budding in endothelial channel. Project oc., No. 2. Apo. oil imm., 2 mm.; camera length, 85 cm.

FIG. 8. *a*. Sporoblast-like body, bone-marrow of leukæmia. Project oc., No. 2. Apo. imm., 2 mm.; camera length, 85 cm.

FIG. 9. Sporoblast-like body. Compen. oc., No. 4. Apo. oil imm., 2 mm.

Fig. 1.

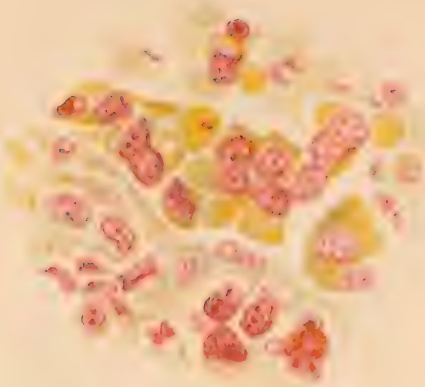

Fig. 2.

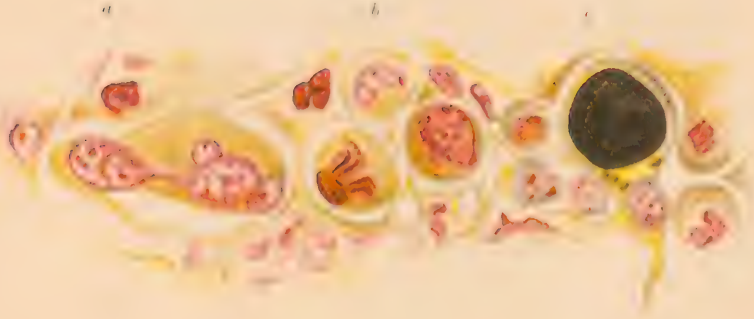

Fig. 3.

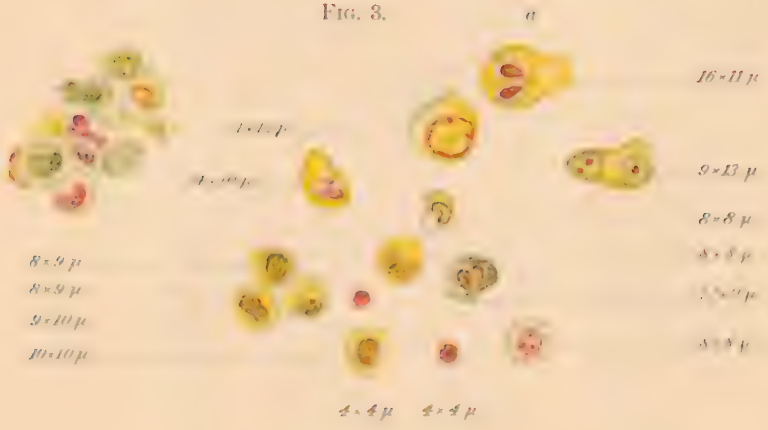

Fig. 4.

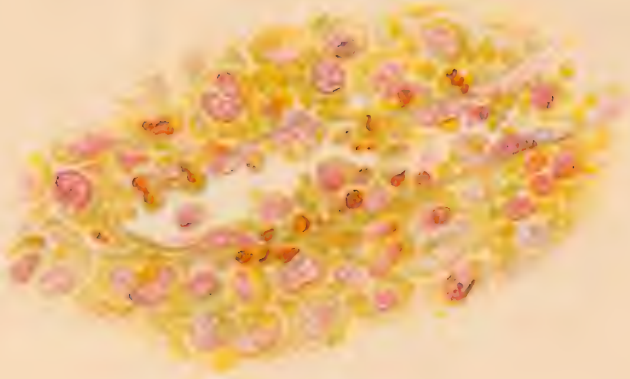

Fig. 9.

Fig. 5.

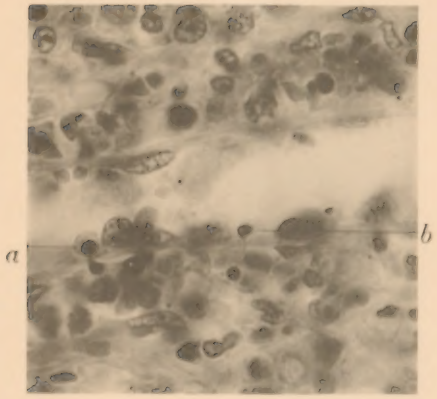

Fig. 6.

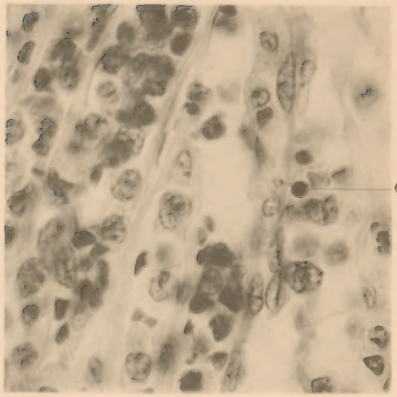

Fig. 7.

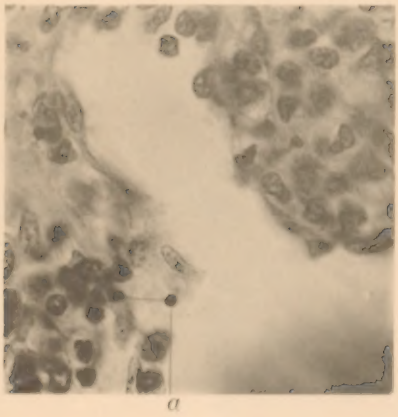

Fig. 8.